Acute exposure to hair bleach causes airway hyperresponsiveness in a rabbit model

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Acute exposure to hair bleach causes airway hyperresponsiveness in a rabbit model. T. Mensing, W. Marek, M. Raulf-Heimsoth, X. Baur. ©ERS Journals Ltd 1998.
ABSTRACT: Ammonium persulphate (APS) and hydrogen peroxide (H_2O_2) are used as oxidants in many industrial processes and are the main constituents of standard hair bleaching products. In a previous study, it was demonstrated that aerosols of APS induce alterations in airway responsiveness.

The present study examined whether exposure for 4 h to a hair bleach composition (containing APS, potassium persulphate and H_2O_2) or H_2O_2 could induce airway hyperresponsiveness and/or an obstructive ventilation pattern in a rabbit model.

Exposure to the aerosols altered neither baseline airway resistance, dynamic elastance, slope of inspiratory pressure generation nor arterial blood pressure and blood gas measurements. Similarly to APS, hair bleach aerosols containing Š10.9 mg·m⁻³ persulphate (ammonium and potassium salt) in air and Š1.36 mg·m⁻³ H_2O_2 in air caused airway hyperresponsiveness to acetylcholine after 4 h of exposure. Aerosolized H_2O_2 (Š37 mg·m⁻³ in air) did not influence airway responsiveness to acetylcholine.

The results demonstrate that hair bleaching products containing persulphates dissolved in H_2O_2 cause airway hyperresponsiveness to acetylcholine in rabbits.
Eur Respir J 1998; 12: 1371–1374.

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In addition to skin diseases, respiratory disorders have been frequently reported among hairdressers and chemical workers exposed to persulphates [1–7]. These substances are inhaled as aerosols or as vapours during the mixing of bleaching powder or paste with the bleaching solution, which contains hydrogen peroxide (H_2O_2), and during application to the hair [8, 9]. In industrial settings, persulphates are used in bleaching processes, for etching of metal surfaces or as polymerization catalysts. H_2O_2 is also used for many bleaching processes and in chemical production processes [10].

A previous study [11] examined the influence of the crude hair bleach component ammonium persulphate (APS) on the development of airway hyperresponsiveness (AHR) in a rabbit model. Exposure was performed twice for 2 h to 500 mg·m⁻³ APS in air. After the first 2 h of exposure to 500 mg·m⁻³ APS, bronchoconstriction induced by acetylcholine (ACh) increased significantly. After another 2 h of exposure, the response to ACh was further increased. After 4 h of exposure to nebulized 50 mg·m⁻³ APS in air, airway responsiveness (AR) was significantly enhanced in a similar manner. No increase in AR to ACh was found during exposure to a concentration of 5 mg·m⁻³ APS in air. During the APS challenge, baseline respiratory and cardiovascular parameters remained unaltered in all groups.

The aim of the present examination was to investigate, in a rabbit model, the influence of a commercial hair bleach with a standard composition and that of H_2O_2 alone on the development of AHR at concentrations near and above the threshold limit volume (TLV).

Methods

Experimental set-up

A detailed description of the rabbit model and the statistical data analysis has been presented in previous papers [12, 13]. Male and female white New Zealand rabbits of the same breed and of similar age and body weight (3.5–4.0 kg) were anaesthetized with 20–30 mg·kg⁻¹ thiopentobarbital sodium (Trapanal®; Byk Gulden, Konstanz, Germany) after premedication with 25 mg·kg⁻¹ ketamine hydrochloride (Ketanest®; Parke-Davis, Berlin, Germany) and 5 mg·kg⁻¹ xylazin (Rompun®; Bayer, Leverkusen, Germany) and placed in a supine position. The level of anaesthesia was kept constant by continuous infusion of 0.2 mg·kg⁻¹·h⁻¹ thiopentobarbital sodium *via* a catheter, inserted into the femoral vein. The body temperature was maintained at 39±0.5°C by means of a thermocontroller, connected to a heating pad. The animals were intubated (3.0 mm i.d.; Mallinckrodt, Athlone, Ireland) and breathed room air spontaneously. All animals were in a healthy condition, free from signs of acute airway infections and had not previously suffered from any known infections.

Recording of respiratory and cardiovascular parameters

Respiratory air-flow (V') was recorded by a Fleisch's head (00; Hugo Sachs, March, Germany) attached to the animal's mouth. Tidal volume (V_T) was obtained by integration of the inspiratory flow signal. Differences in oesophageal pressure ($ýP_{oes}$) were measured using a catheter

inserted in the oesophagus connected to a pressure transducer. Dynamic elastance (E_{dyn}) was calculated from ýP_{oes}/V_T. By catheterization of the femoral artery, cardiovascular parameters were measured and small blood samples (about 0.4 mL) were collected to examine blood gases and correlated acid–base parameters. Data were recorded on a polygraph (Graphtec Linearcorder WR 3310; Graphtec, Tokyo, Japan) and, after analogue/digital conversion of the measured signals, digitized on a personal computer.

Airway hyperresponsiveness in in vivo *experiments*

Changes in airway response to aerosols of 0.2% and 2% ACh solutions in saline, generated by a commercial nebulizer (Pari, Clinic II; Starnberg, Germany), were investigated. Solutions (0.13 mL) were nebulized in 5.7 L room air·min^{-1} and stored in a reservoir bag. The particles had a diameter of 0.5–5.5 µm. During the challenge tests, animals inhaled for 1 min with a mean of 1.1±0.3 L from the ACh aerosol [12], which corresponded to a total dose of 0.05 and 0.5 mg ACh, respectively (0.2% and 2.0% solution).

Changes in airway responses were measured when respiratory and cardiovascular parameters were constant. At the beginning, inhalation of aerosolized 0.2% ACh solution did not change baseline values of E_{dyn} in healthy rabbits. Application of nebulized 2.0% ACh solution caused a rise in E_{dyn} of 50–150% of the baseline value. Attention was focused on the responses to aerolized 2.0% ACh solution, before and after exposure to irritating agents. The responses to ACh were transient, lasting for <15 min.

Each group, consisting of eight or nine rabbits, inhaled one concentration of hair bleach or H_2O_2, respectively. All agents were tested at three different concentrations. ACh challenge tests were performed before and after 2 and 4 h of exposure. The chemicals $(NH_4)_2S_2O_8$, $K_2S_2O_8$, and H_2O_2 (30%) were obtained from Merck (Darmstadt, Germany).

Control group A. In control group A, animals (n=9) were exposed twice for 2 h to aerosolized saline. The AR to ACh was tested before and after 2 and 4 h of exposure.

Exposure to hydrogen peroxide (groups B–D). Hydrogen peroxide was diluted in saline. The groups (n=8 each) were exposed to concentrations of 37 (group B), 7.5 (group C) or 0.75 mg·m^{-3} (group D) H_2O_2 in air for 4 h. The estimated (from concentration and minute volume) inhaled doses were 7.1, 1.4 and 0.1 mg, respectively.

Exposure to hair bleach solution (groups E–G). Three groups of animals (n=8 each) were exposed to three different concentrations of a nebulized hair bleach (dilutions of a commercial stock solution with standard composition). When nebulized, the total aerosol concentrations were 1,200, 120 or 12 mg·m^{-3} in air (groups E–G), corresponding to the inhalation of 230, 23 or 2.3 mg hair bleach in 4 h, respectively (for composition see table 1). The standard hair bleach was dissolved in saline and contained only bleaching agents (*i.e.* APS, potassium persulphate (KPS) and H_2O_2) without compounds for stabilization or perfume.

Table 1. – Concentrations (mg·m^{-3} in air) of the individual compounds in the inhaled aerosols

	Hair bleach composition			Crude substances	
	H_2O_2	APS	KPS	H_2O_2	APS [11]
Dilution 1:10	136	410	678	37	500
1:100	13.6	41	67.8	7.5	50
1:1000	1.36	4.1	6.78	0.75	5.0
TLV [14]				1.4	
TLV [15]					5

H_2O_2: hydrogen peroxide; APS: ammonium persulphate; KPS: potassium persulphate; TLV: threshold limit value.

Statistical analysis. The data represent the mean±SD of n=8 or n=9 of the respective group. The significance of the differences between the ACh response before and after exposure to the chemical compounds was tested by Students' paired t-test [14]. Differences were considered significant for p<0.05.

The experiments were performed with the written consent of the ethical commission for animal experiments.

Results

Control group A

Group A was exposed twice for 2 h to saline aerosols. The first inhalation of nebulized 2.0% ACh solution at the beginning increased E_{dyn} by about 100% of the baseline value from 2.5±0.3 to 5.1±1.1 kPa·dL^{-1}. No significant response was found to aerosolized 0.2% ACh solution. Two 2-h exposure periods to saline aerosols did not influence the ACh responses (fig. 1).

Influence of hair bleach on the development of airway hyperresponsiveness

During exposure, the baseline respiratory and cardiovascular parameters remained unaltered. Exposure to an aerosol containing 1,200 mg·m^{-3} of a hair bleach solution in air (group E) caused a significant increase in the response to 2.0% ACh after 2 and 4 h of exposure (p<0.05 and p< 0.005, respectively). In the groups exposed to 12 mg·m^{-3} (group H) or 120 mg·m^{-3} (group G), AR to ACh increased significantly after 4 h (p<0.05) (fig. 2).

Fig. 1. – Changes in dynamic elastane (ýE_{dyn}) after challenge with 2.0% acetylcholine hydrochloride before and 2 h and 4 h after exposure to saline (n=9).
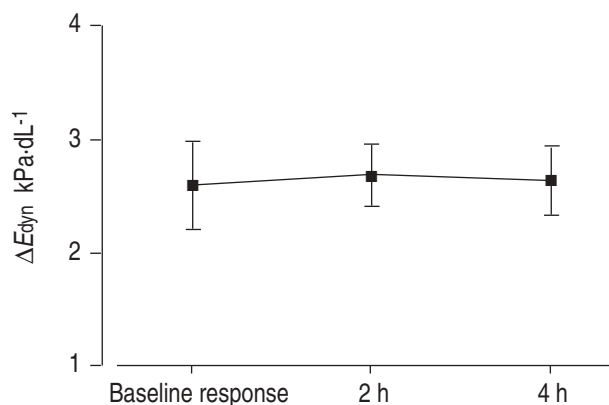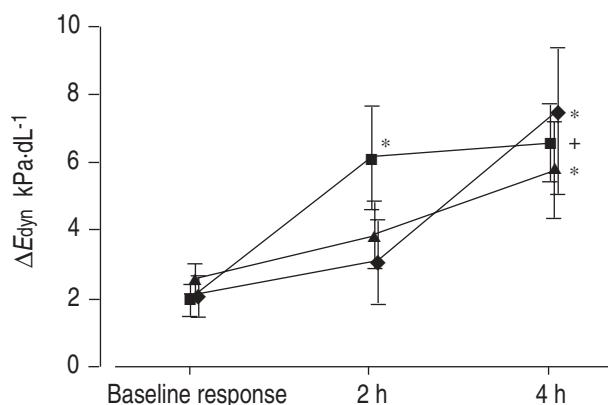
Fig. 2. – Changes in dynamic elastance ($\acute{y}E_{dyn}$) after challenge with 2.0% acetylcholine hydrochloride before and 2 h and 4 h after exposure to three different concentrations of a hair bleach aerosol (◆: 12 mg·m⁻³; ▲: 120 mg·m⁻³; ■: 1,200 mg·m⁻³) (n=8). *: p<0.05, ⁺: p<0.005.

Influence of hydrogen peroxide on the development of airway hyperresponsiveness

Baseline values of respiratory mechanical parameters were not significantly altered during and after H_2O_2 aerosol inhalation. The responses of E_{dyn} after ACh challenge were not significantly altered after exposure to the three different H_2O_2 concentrations (groups C–E; fig. 3). However, after exposure to 37 mg·m⁻³ H_2O_2 in air, there was a small but nonsignificant trend towards an increased contractile response to 2% ACh.

Discussion

This study examined the influence of a standard hair bleach composition and the main constituents of hair bleach on the development of AHR in rabbits *in vivo*. APS [11] as well as the standard hair bleach (12 mg·m⁻³), but not H_2O_2 (37 mg·m⁻³), caused significant increases in airway contractile responses to ACh.

In the control group, exposed to saline aerosols, AR remained stable. In previous experiments, it was demonstrated that APS at concentrations Š50 mg·m⁻³ in air caused AHR, after an exposure of only 4 h [11]. However, exposure to hair bleach containing 11 mg·m⁻³ persulphate
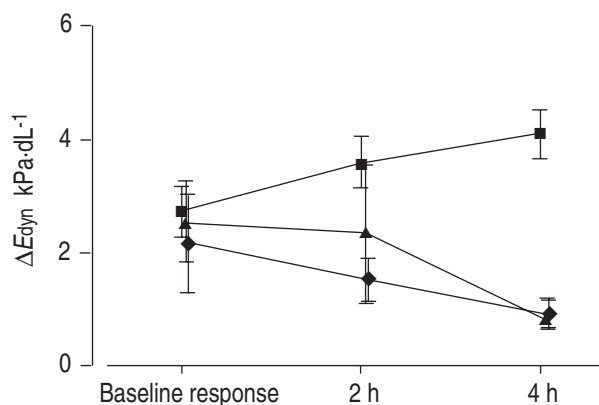

Fig. 3. – Changes in dynamic elastane ($\acute{y}E_{dyn}$) after challenge with 2.0% acetylcholine hydrochloride before and 2 h and 4 h after exposure to 0.75 (◆), 7.5 (▲) and 37 mg·m⁻³ (■) H_2O_2 (n=8).

(4 mg·m⁻³ APS) also caused AHR after 4 h of exposure. Moreover, 5 mg·m⁻³ pure APS resulted in an increase in eosinophil-released mediators in bronchoalveolar lavage (BAL) fluid in rabbits [11, 15]. It seems likely that chronic exposure to APS at a concentration near the TLV (5 mg·m⁻³), which may occur in some working environments (*e.g.* hairdressing salons, APS production), might cause airway inflammation. MERGET *et al.* [7] measured APS concentrations of 3.6 mg·m⁻³ in a chemical production plant of persulphates.

The present results are partially in accordance with the experiments of LAST *et al.* [16]. These authors demonstrated that exposure to an APS concentration of 4 mg·m⁻³ for 7 days, 23.5 h·day⁻¹, caused a decreased body weight and an increased fresh lung weight. The discrepancy with the present results at 5 mg·m⁻³ might be due to the longer exposure effects not evaluated in these experiments.

The persulphate concentrations in the hair bleach mixture and in the pure APS aerosol were different, so it is difficult to compare the two groups. However, no statistical difference can be obtained between the groups exposed to pure persulphate or hair bleach.

In the animals exposed to different concentrations of the strong oxidant H_2O_2, no enhanced responses to ACh were found. The reason for this outcome has not yet been clarified. The presence of glycoproteins or enzymes such as catalase or glutathione peroxidase, which metabolize H_2O_2 in the lung, may prevent the damage of airway epithelium function [17, 18] and hence the development of AHR. Although there was a trend towards an increased responsiveness to 2% ACh after exposure to 37 mg·m⁻³, H_2O_2 had no effects on AR in the concentrations used in hair bleach solutions. An effect of H_2O_2 at higher concentrations cannot be excluded. Moreover, the choice of the species and the model may influence the effect of H_2O_2 [19–21].

The hair bleach solutions contained neither perfumes nor stabilizing agents, the presence of which may vary in different commercial products, to avoid any influence of these compounds. Because of the very high concentration of persulphates in the hair bleach mixture, diluted solutions containing concentrations of the crude substances near the TLV were used.

The basic mechanisms of APS and hair bleach-induced AHR are still unclear and need further investigation. However, several mechanisms of action could explain the effect of persulphates on AR.

Although persulphates are stable in aqueous solutions, a small proportion decays to oxygen radicals [22]. A reaction with organic materials is mediated by the radicals ·SO_4^- or ·OH. Inhaled oxidants damage epithelial cells [23, 24], and this may result in a loss of prostanoid inhibitory factors [25]. Oxidation of unsaturated fatty acids would result in an increased concentration of prostaglandins (PGE_2), which could also influence airway smooth muscle tone [23, 25].

Induced epithelium damage would favour airway contractile mechanisms. This is supported by the results of MUNAKATA *et al.* [26], who showed that AR increased in the absence of an intact epithelium. Another explanation for the development of AHR may be the release of histamine from mast cells, as demonstrated by PARSONS *et al.* [27].

Further investigations into the cell and mediator profile in the BAL fluid demonstrated the participation of inflammatory cells and their mediators in the development of

AHR after exposure to the examined substances in the lowest concentrations [15]. It is well known that eosinophils and their mediators, such as eosinophilic peroxidase, major basic protein and leukotrienes C_4, D_4 or E_4, contribute to the development of AHR. A release of reactive oxygen species from inflammatory cells might cause damage to the epithelial cells [28]. Koller et al. [29] showed that APS may increase the generation of leukotrienes from human neutrophil granulocytes.

In summary, exposure to hair bleach containing about 10 mg·m^{-3} persulphates increased airway hyperresponsiveness after 4 h. Hydrogen peroxide in a 26-fold concentration of the German threshold limit value neither altered respiratory parameters nor influenced airway hyperresponsiveness to acetylcholine. These results confirm the initiating toxic character of persulphates and hair bleach in concentrations near and above the threshold limit value of ammonium persulphate.

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